IEP Proposal
Quality Assurance Quality Control
Checklist

Program Element Title:
Historical Benthos Biomass at Long-Term IEP EMP Benthos Monitoring Stations

Principal Investigators:
Karen Gehrts (DWR), (916) 227-0438, kagehrts@water.ca.gov
Anke Mueller-Solger (DWR), (916) 227-2194, amueller@water.ca.gov

Collaborators:
Heather Peterson (hapeters@usgs.gov)
Jan Thompson (jthompso@usgs.gov)
Marc Vayssieres (marcv@water.ca.gov)
Wayne Fields, Hydrozoology

Proposed Reviewers:
Dr. Terry Short, USGS (tmshort@usgs.gov)
Dr. Alan Jassby, UCD (adjassby@ucdavis.edu)
Zachary Hymanson, CALFED (zachary@water.ca.gov)

Total IEP Special Studies Funding Requested: $34,265 for calendar year 2004 and **$32,517 for 2005** (or $62,746 for 2004 and $61,368 for 2005 if hiring freeze is in effect)

I. Program Element Management

A. Program Element Description/Problem Definition

1. History or Background

Over the past three decades, the IEP Environmental Monitoring Program (EMP) has recorded benthos community composition and abundance at a total of 22 sites in the upper San Francisco Estuary (SFE; Figure 1) which includes the Sacramento-San Joaquin Delta, Suisun Bay, and San Pablo Bay. This has permitted the detection of exotic invaders and allowed for investigations of benthos responses to water quality changes and project operations (Markmann 1986, Hymanson et al. 1994, Cohen and Carlton 1995). However, the EMP has been criticized with increasing frequency for not measuring benthic biomass in addition to abundance and community composition (Hymanson et al. 1994, IEP EMP Benthos SAT review 2001, IEP EMP SAG review 2002). Here, we explore the rationale for monitoring benthic biomass and propose to a) estimate the biomass associated with historical species abundance data at four long-term EMP benthos monitoring sites (circled in Figure 1) from archived samples using a simple "wet-weight method" for use in a community analysis proposed by Peterson et al. and b) examine which biomass method is most suitable for future benthic monitoring, analyses, and special studies.

Biomass and associated organic carbon concentrations are universally used as the basic "currency" for ecological analyses and modeling, including carbon budget estimation and food web analyses and modeling. These types of analyses are necessary to develop conceptual models and hypotheses about factors and mechanisms responsible for ecological patterns and trends such as those observed by the EMP and other IEP monitoring programs. In a complex ecosystem such as the SFE, many natural and anthropogenic factors interact to produce the observed trends.

The EMP and other IEP programs are tasked with detecting and tracking the environmental impacts associated with a specific anthropogenic factor, the operation of the California State Water Project and the Federal Central Valley Project. The resulting data is intended to help resource managers and project operators protect beneficial uses, including several threatened and endangered fish species occurring in the upper SFE. This necessitates distinguishing project operations from other important factors and mechanisms affecting the ecology of the upper SFE (e.g., benthic grazing, species invasions) and thus requires a comprehensive monitoring program design incorporating variables lending themselves to both concentration/abundance and flux/transfer analyses.

In addition to classical inorganic and physical water quality variables, the EMP has monitored variables associated with bulk organic matter (OM) as well as primary producer (phytoplankton) composition, abundance and biomass (chlorophyll *a*), and primary consumer (zooplankton, benthos) composition and abundance throughout its existence. These measurements are important in assessing the impacts of project operations on food quantity and quality for higher trophic levels and on biogeochemical cycling of elements and compounds, including nutrients and contaminants. For example, on a Delta-wide basis, EMP data has revealed strong impacts of project operations on OM concentrations, especially during dry years when the projects represent the dominant OM export route during all seasons (Jassby and Cloern 2000). OM forms the nutritional basis for all consumers in the upper SFE, and one aspect of protecting beneficial uses is to assure food supplies of sufficient quantity and quality. Below we summarize recent work examining the interplay between bulk OM, bioavailable OM (phytoplankton), project operations, benthic organisms, and other factors. In addition to its role in the food web, OM is also implicated in the formation of toxic drinking water disinfection byproducts such as trihalomethanes (CDWR 1994) and oxygen depletion in the San Joaquin River near Stockton (Ritchie 2002). As we explain below, both water project operations and benthic organisms affect these processes through their impact on OM concentrations.

While detrital OM is usually abundant in the upper SFE, recent research has shown that the nutritionally superior phytoplankton fraction of OM is often too scarce to allow for optimal consumer growth (Sobzack et al. 2002, Mueller-Solger et al. 2002). Moreover, phytoplankton biomass and primary production in the Delta have declined from 1975 to 1995 by 59% and 43%, respectively (Jassby et al. 2002). At the same time, declines of many native consumer species have been observed by EMP and other IEP monitoring and in many cases attributed to increasing food shortages (Bennett and Moyle 1996, Kimmerer and Orsi 1996, Orsi and Meecum 1996, Kimmerer and Penalva 2000). Using 1975 - 1995 EMP data, Jassby and Cloern (2000) and Jassby et al. (2002) identified major sources and sinks for OM and phytoplankton in the Delta. They estimated that, on average, 32% of all Delta phytoplankton is flushed from the Delta through diversions (9% of phytoplankton supply), outflow to the SF Bay (9%), and water project operations (14%). The remaining 68% are consumed or buried within the Delta. Project operations thus have a substantial impact on food supplies for consumers in the Delta and adjacent downstream areas such as the ecologically important Suisun Marsh. However, "consumption and burial" represent an even larger phytoplankton sink. Moreover, while water project exports are an important element in Delta-wide carbon and phytoplankton budgets, they do not appear to be a primary mechanism underlying phytoplankton variability in the Delta. Instead, Jassby et al. (2002) were able to show that consumers were responsible for the main mode of variability found in the long-term EMP phytoplankton biomass data set, followed by climate (flow) effects and turbidity fluctuations due to dams, etc. The most striking consumer impact was the consistent drop in phytoplankton biomass during June - November associated with the introduction and rapid spread of the clam *Potamocorbula amurensis* in 1987. Jassby et al. also hypothesized that another, earlier benthic invader, the clam *Corbicula fluminea*, may be behind a long-term phytoplankton decline during winter, but the existing EMP benthos data is insufficient to test this hypothesis at this time.

In summary, while project operations substantially affect the mass balance of food resources in the Delta, consumers, and in particular benthic macroinvertebrates, may have a larger impact on inter- and intraannual variability of food supplies. However, reliable quantification of Delta-wide carbon losses due to benthic grazing has not been possible due to the **lack of benthic biomass monitoring data** for the Delta. As we will explain below, population biomass estimates based on organism abundance and a constant conversion factor for each taxon (gleaned from the literature or obtained in a special study) are insufficient approximations for many benthic species. In consequence, project impacts on food supplies within the Delta cannot be distinguished from benthic grazing impacts, nor can benthic grazing be distinguished from grazing by other organisms. The EMP thus falls short of fulfilling its mission. Because of the lack of benthos biomass data, it is currently equally impossible to identify project operation effects on benthos growth, grazing, and metabolic rates and needs. Besides their trophic implications, these rates are also important in biogeochemical cycling, transfers, and transformation of important elements and compounds such as the disinfection byproducts and dissolved oxygen mentioned above and heavy metals such as Selenium and Mercury (Luoma and Linville 1995). Fortunately, the EMP has archived benthos samples dating back to 1977 which can be used for biomass estimation using a simple wet-weight method. We propose to obtain historical biomass data for four EMP sampling stations (Figure 1) slated for in-depth benthic community analysis by Peterson et al. We will also explore which biomass estimation method is most suitable for future incorporation into routine EMP benthos monitoring and for estimating historical benthos biomass at other EMP sites. We will make the historical biomass data immediately available for the retrospective benthos analyses proposed in the sister project by Peterson et al. and for other analyses through the IEP relational B-DAT database and its web interface.

2. Purpose of Program Element in Explicit Terms

a) Objectives, questions, hypotheses, and tasks

The objective of the proposed study is to obtain historical biomass data for four EMP sampling stations for immediate use in a proposed retrospective analysis by Peterson et al. and to evaluate biomass estimation methods for potential future incorporation into the EMP benthos element and historical biomass estimation at other EMP benthos sites. As a byproduct, a reference collection for benthic species encountered by EMP sampling will be established during the course of this work. The four stations chosen (Figure 1) are the IEP EMP benthic monitoring sites with the longest continuous sampling records (1977 - present). These are the same stations targeted by a proposed sister study, the retrospective analysis of leng-term benthic community data proposed by Peterson et al for 2004 IEP funding.

Ideally, benthic biomass (as dry tissue weight) is estimated by measuring the length of each animal in each sample and converting these lengths into weights based on equations of weight predicted from length. These equations are determined, during each season at a minimum, with live animals and throughout the duration of a study (Ricciardi and Bourget 1998, and Appendix, biomass method b)). Overall this method is very labor-intensive, but delivers highly accurate biomass estimates. Unfortunately it cannot be used for biomass estimates in historical, preserved samples such as the archived EMP samples due to the need for unpreserved tissue.

Another, much simpler method is to estimate benthic organism biomass using a constant length-to-weight conversion factor (e.g., an average - see Appendix, biomass method a)). However, in contrast to many planktonic species, this often results in large over- or underestimates of benthic biomass (Figures 2 and 3). The main reason for this is the large temporal variability in size and biomass of many benthic species depending on their developmental stage and environmental conditions. This variability cannot be accounted for by a single, constant conversion factor per species. For example, if abundance is multiplied by average species biomass, the biomass results overestimate the actual population biomass when many small juvenile organisms are present, and underestimate the actual biomass when large adults are dominating the population. This then leads to large over-or underestimates of grazing rates (e.g. grazing of one versus 10 m water column) and erroneous associated conclusions about the importance of benthos grazing and the availability of food supplies for other primary consumers, precursors for toxic disinfection byproducts, etc. Figures 2 and 3 illustrate this problem with EMP and USGS data. Figure 2 also shows different lengths of the over-and underestimation periods during two different years, indicating variability in recruitment cycles within and between years. This common and often substantial intraannual variability may prevent generalized correction for differences in benthos biomass by season or month. For more details about these figures, see Appendix.

Because of the large and inconsistent biomass variability in benthic species over various time scales, it thus appears necessary to obtain benthos biomass data along with abundance data. In this study, we propose to measure benthic species biomass in archived, preserved benthos samples from the four most consistently monitored and most long-term EMP benthos stations using a simple "wet-weight" technique (method details in II B., below). We will also explore how this technique compares to the "dry-tissue-weight" technique and the "constant factor" techniques described above and in the Appendix. In contrast to these two methods, the wet-weight technique has not been previously used for benthos biomass estimation in the upper SFE and a comparison using EMP data is thus not possible at this time. However, this technique has been successfully applied in various marine, estuarine (Ricciardi and Bourget 1998) and freshwater systems (Wong et al. 1998). Finally, we will explore which method and exact procedure would be best for incorporation into the EMP benthos program element and for potential estimation of biomass in additional historical samples from other sites.

The hypotheses to be tested may be stated as follows. Please note that hypothesis testing only pertains to the examination of biomass estimation techniques, not to the other, larger part of this study, i.e. obtaining historical biomass estimates.

a. The "wet-weight" method for estimating benthic biomass delivers results that are more similar to the (desirable) results obtained using the "dry-tissue-weight" technique and less similar to the (undesirable) results obtained using the "constant factor" technique, and are overall of sufficient accuracy for EMP purposes.
b. Published wet-weight to dry-weight conversion equations for individual taxa (phyla, orders, or families) used in the "wet-weight" method deliver satisfactory results for common benthic species in the SFE.
c. Estimating historical biomass in one sample per site and sampling event rather than in multiple replicate samples is sufficient to detect changes in benthos biomass and provide data sufficient for meaningful ecological analyses of growth and grazing rates, etc. (Processing only one replicate significantly reduces labor hours. Also, only one of tree replicates was kept and archived before 1983.)

These hypotheses will be tested through the following tasks:

a. Compare all three methods for at least one species for which dry-weight data exists (e.g., expand the *Potamocorbula amurensis* and *Corbicula fluminea* comparisons shown in Figure 2 and 3 to include new wet-weight method data and simple statistical comparisons of results (ANOVA, regression)).
b. Test the validity of wet-weight to dry-weight conversion equations from literature sources on at least one SFE species (e.g. *Potamocorbula amurensis)* for which we can compare biomass estimates using the "wet-weight" method with previously measured tissue dry-weights, or collect additional samples for the purpose of examining these conversion equations.
c. For several seasons of several historical years (at minimum, one wet, one dry, and one normal hydrologic year), estimate benthos biomass using the wet-weight method for all preserved replicate samples per site and compare the results with the results for one randomly chosen replicate per site. Conduct a power analysis to determine the effects of different degrees of replication on detection of biomass changes. Closely work with the proposed sister project by Peterson et al. to determine the usefulness of biomass data from only one replicate for ecological analyses.

b) Determination of success

The study will be successful if by the end of the two-year study period, (1) biomass data has been obtained for historical IEP EMP benthos samples from four sites, if this data has been stored on the EMP server and made accessible through the IEP-EMP/BDAT data base; (2) a reference collection has been established; and if (3) the method evaluation outlined in tasks a. - c., above, has been completed and resulted in an IEP newsletter article and technical report containing and discussing results and recommending a biomass estimation method for incorporation into the EMP as well as in a presentation at the IEP Asilomar workshop.

3. How will data and program information elements be used?

In general, the EMP data has historically been used to gain new insights into the ecology of the San Francisco Estuary and estuarine environments, to detect and track exotic species invasions and their impacts, and to provide baseline data for resource management. These insights are fundamental to the development of novel, sustainable management practices. The data and information gained from this program element will be used to improve the EMP benthic element as recommended in the recent EMP review and thus serve the public by producing more relevant baseline data about the composition, prevalence, and ecological role of benthic macroinvertebrates in the San Francisco Estuary.

Specifically, biomass data from this study will be provided as soon as it becomes available for long-term community analysis in the proposed sister study by Peterson et al. and for use by other interested IEP researchers and the public via the BDAT data base and web interface. Notes, pictures, and other observations from this study as well as the reference collection established while processing the historical samples will also benefit the proposed "Benthos Bio Guide" project and, through it, a wider audience (see sister proposal by Messer et al.). Data and other information will be stored in the EMP database on the EMP server and made accessible via the IEP data vaults BDAT web interface and possibly the proposed IEP Bio Guide web interface. Results from tasks a. - c., above, will be published in the IEP newsletter article along with recommendations for routine benthic biomass estimation by the EMP. Another benefit of this and the proposed sister studies is an increase in EMP staff expertise in benthos ecology and taxonomy. To date, all EMP benthos sample processing has been conducted by Wayne Fields of Hydrozoology, the regional expert on benthos taxonomy. Wayne Fields will teach and advise EMP staff in benthos taxonomy during this study. In the course of this study, Wayne Fields will thus transmit much of his expertise to EMP staff and help establish the EMP benthos reference collection to assure the long-term quality and consistency of EMP benthos data. This work will be conducted in close cooperation with the "Benthos Bio Guide" sister project proposed by Messer et al.

4. How may this data and information be used by other current studies or future work?

As mentioned above, the biomass data and ancillary information will immediately benefit the proposed IEP studies by Peterson et al. and Messer et al. and through these studies, benefit the EMP, IEP, and the wider community of interested scientists and resource managers. The results of the method evaluations will benefit future IEP EMP monitoring and other studies involving benthos sampling and ultimately lead to better data for detecting and managing project operation impacts. Furthermore, data and information collected in this study will be made available for use by other scientists and resource managers through the IEP database. Historically, EMP data has been used extensively by scientists from other agencies and Universities and resulted in valuable insights such as the new understanding about fate and sources of organic carbon and phytoplankton mentioned above.

5. What are the biological implications of the program element?

This study will improve our understanding of the role of benthic organisms in the upper portion of the San Francisco Estuary by allowing for better assessments of secondary production, transmission of environmental pollutants, approximations of the contribution of each species to community biomass and changes in species condition over time in reaction to water project operation and other factors.

6. Has this proposal been submitted elsewhere for funding or do you plan to submit it elsewhere for funding?

This proposal has not been submitted elsewhere for funding and we do not plan to submit this proposal elsewhere for funding.

B. Project Resource Needs

Total budget and total special studies funds requested from IEP:

The total budget for this project is $168,678 without continued State hiring freeze or $226,010 with the current State hiring freeze still in effect. Of these, $101,896 will be covered by the EMP through the involvement of DWR-EMP staff (Anke Mueller-Solger, Karen Gehrts). The total **special studies funding requested from IEP for this two-year study is $66,782 without State hiring freeze or $124,114 with State hiring freeze in effect** for new temporary personnel and supplies. Projected activity rates for fiscal years 2003, 2004 and 2005 shown in the Personnel Table, below, were used to calculate labor costs. These rates include overhead and benefits. As shown in greater detail in the personnel and budget tables, below, without hiring freeze, **we are requesting $34,265 for calendar year 2004 and $32,517 for calendar year 2005. With hiring freeze in effect, we are requesting $62,746 for calendar year 2004 and $61,368 for 2005.**

Budget considerations and itemization:

The State of California is currently enforcing a hiring freeze. It is our understanding that the freeze may remain in effect until July 2004 or longer. If the hiring freeze will indeed be enforced during this project and we thus have to delay or cancel hiring a Fish and Wildlife Scientific Aid I through DWR, we propose the following alternative plan: Our collaborators at the USGS in Menlo Park have agreed to hire or assign an equivalent temporary employee to fulfill the Fish and Wildlife Scientific Aide position in this project, if necessary. Upon receiving word that this project is to be funded we are fully prepared to begin the contracting process with the USGS. The USGS cost figures, including benefits for a GS-5 (comparable to Scientific Aide) are $26.15, $27.53, and $28.91 for fiscal years 2003, 2004 and 2005 per hour. In addition, there will be USGS overhead costs. This will increase the budgetary needs for this project. Upon confirmation of the length of the hiring freeze we will adjust our budget accordingly. We are also open to other suggestions/options (e.g. hiring through the Science Consortium) if such alternatives become available.

Equipment: No additional sampling equipment or boat time will be needed for this project, as historical and routine EMP benthos monitoring samples will be used. DWR-DES owns a balance and microscopes suitable for this project.

Supplies:

Supplies Needed	Cost
Miscellaneous Supplies (ethanol, vials, forceps, etc…)	$ 7,000
TOTAL	**$ 7,000**

Personnel:

Funding to be covered through the EMP:					
Position	**% Time Commitment**	**Hours for Project**[1]	**Activity Rate**[2]	**Cost**	**Task**
Staff Scientist (Anke Mueller-Solger)					
1/04 to 6/04	10%	100	$62.02	$6,202.00	Supervision and Data Analysis
7/04 to 12/04	10%	100	$68.22	$6,822.00	Supervision and Data Analysis
1/05 to 6/05	10%	100	$68.22	$6,822.00	Supervision and Data Analysis
7/05 to 12/05	10%	100	$75.04	$7,504.00	Report Writing and Presentations
			Total Cost	**$27,350.00**	
Position	**% Time Commitment**	**Hours for Project**[1]	**Activity Rate**[2]	**Cost**	**Task**
Environmental Scientist B (Karen Gehrts)					
1/04 to 6/04	30%	300	$62.02	$18,606.00	Data Collection, Entry, and Analysis
7/04 to 12/04	30%	300	$68.22	$20,466.00	Data Collection, Entry, and Analysis
1/05 to 6/05	30%	300	$68.22	$20,466.00	Data Collection, Entry, and Analysis
7/05 to 12/05	20%	200	$75.04	$15,008.00	Report Writing and Presentations
			Total Cost	**$74,546.00**	
Total EMP Personnel Contribution (no additional IEP funds requested):				**$101,896.00**	
2004 Special Studies Personnel Funds Requested From IEP					
Position	**% Time Commitment**	**Hours for Project**[1]	**Activity Rate**[3]	**Cost**	**Task**
Consultant (Wayne Fields, Hydrozoology)					
1/04 to 6/04	5%	50	$80.00	$4,000.00	Consulting on Reference Collection
7/04 to 12/04	5%	50	$85.00	$4,250.00	Consulting on Reference Collection
1/05 to 6/05	5%	50	$85.00	$4,250.00	Consulting on Reference Collection
			Total Cost	**$12,500.00**	
Position	**% Time Commitment**	**Hours for Project**[1]	**Activity Rate**[4]	**Cost**	**Task**
Scientific Aide (new hire)					
1/04 to 6/04	50%	475	$22.57	$10,720.75	Hypothesis Testing and Data Collection
7/04 to 12/04	50%	475	$24.83	$11,794.25	Hypothesis Testing and Data Collection
1/05 to 6/05	50%	475	$24.83	$11,794.25	Hypothesis Testing and Data Collection
7/05 to 12/05	50%	475	$27.31	$12,972.25	Report Writing and Presentations
			Total Cost	**$ 47,281.50**	
Total Personnel Funds Requested From IEP without State Hiring Freeze:					**$59,781.50**
To Be Hired Only if State Hiring Freeze is Still In Effect (instead of DWR Scientific Aide):					
Position	**% Time Commitment**	**Hours for Project**[1]	**Activity Rate**[4]	**Cost**	**Task**
GS 5					
1/04 to 6/04	50%	475	$26.15	$12,421.25	Hypothesis Testing and Data Collection
7/04 to 12/04	50%	475	$27.53	$13,076.75	Hypothesis Testing and Data Collection
1/05 to 6/05	50%	475	$27.53	$13,076.75	Hypothesis Testing and Data Collection
7/05 to 12/05	50%	475	$28.91	$13,732.25	Report Writing and Presentations
			Subtotal	$ 52,307.00	
			[5]**Total Cost**	**$ 104,614.00**	
Total Personnel Funds Requested From IEP With State Hiring Freeze In Effect:					**$117,114.00**

[1]Anke Muller-Solger will be serving as the Staff Scientist and Karen Gehrts will be serving as the Environmental Scientist B for this project. The Cost figures above for Staff Scientist and Environmental Scientists B were based on a 2000 hour work year.

[2] Activity Rates for the Staff Scientist, Environmental Scientist B and the Scientific Aide were provided by DWR's accounting office and the Activity Rates for the GS 5 were provided by the USGS accounting office.

[3] Consultant Activity Rates were obtained from Wayne Fields at Hydrozoology and are based on a 2000 hour work year.

[4]The Scientific Aide will be hired upon receiving the funding for this project. However, if DWR's hiring freeze is still in effect when this project is scheduled to begin, the GS 5 will be hired. The Cost figures for these positions were based on a 1900 hour work year.

[5]The Total Cost listed under the GS 5 position includes USGS estimated overhead.

C. ESA Considerations

This program element will not result in the "taking" of any state or federally listed threatened or endangered species.

D. Due Dates and Products

1.<u>Program element timeline, deliverables and completion dates</u>.

Study Duration:

The study duration is two calendar years, 2004 and 2005. These two calendar years encompass three fiscal years (2003, 2004 and 2005).

Deliverables and completion dates:

Task and Personnel Effort	**Product and Completion date**
Processing (sorting and weighing) historical samples, data entry, and assembling reference collection: DWR Scientific Aid (or USGS technician) (50% - sample processing) Karen Gehrts (10% - data management/ entry, some sample processing, reference collection) Wayne Fields (5% - taxonomic consultation, reference collection) Anke Mueller-Solger (2% - supervision)	Sample processing: D7: September 2004; D41A: January 2005; D4: April 2005; D28 A: August 2005; Data base, including uploading to the web: September 2005. Reference Collection: September 2005
Method evaluations (hypothesis testing) Karen Gehrts (10-20%) Anke Mueller-Solger (3%)	Completed tasks a-c, above. June 2005
IEP report, newsletter article, and presentation at the IEP meeting in Asilomar Karen Gehrts (0-10%) Anke Mueller-Solger (5%)	Report and article drafts: September 2005; final drafts: December 2005; Presentation: February 2006

Other products include increased benthic expertise among EMP staff and notes, pictures, and other observations of benefit to the proposed “Benthos Bio Guide of the San Francisco Estuary” (Messer et. al.) and/or used in the EMP benthos metadata files.

2. <u>Will any databases be created for or added to for this program element?</u>

We do not expect to create a new database for the historical biomass data collected during this study. We will modify the current EMP benthic database housed on the EMP server in the DWR-DES Environmental Compliance and Evaluation Branch to accommodate data collected during this study. This will ensure that data are compatible and will thus lead to further and more in-depth analysis of the collected data. We will also make the data available over the IEP BDAT database and its web data interface. The much less extensive data from the proposed methods evaluations will be stored in spreadsheets or in a simple Microsoft Access database on the EMP server. These data will be made available to others upon request.

3. Will the data be uploaded to the IEP server and if so, when?

Yes, the historical biomass data will be uploaded onto the IEP server monthly in conjunction with the EMP Benthos abundance data uploads. Uploading should be completed by September 2005.

II. Program Element Measurement and Data Acquisition

A. Sample Site Selection

We propose to process historical samples from EMP sites D7 (Grizzly Bay), D4 (Collinsville), D41A (San Pablo Bay) and D28A (Old River). These sites are the IEP EMP benthic monitoring sites with the longest continuous sampling records and suitably preserved samples have been archived for these sites. Moreover, previous biomass measurements for clams using the "dry-tissue-weight" method exist for D7 and D41A (J. Thompson, pers. comm.) and can be used for our method evaluations. The four sites are located along the axis of the estuary spanning the brackish to freshwater habitats and representing shallow embayments as well as cross-channel locations, see Figure 1. They are the same sites targeted for long-term analyses by Peterson et al.

B. Sampling, Sample Processing and Analysis

The historical samples which will be used in this study were collected and processed using standard EMP methods as follows:

Benthic samples are collected from a boat with a Ponar Dredge with a sampling area of 0.053 m2. The contents of each grab sample are carefully washed into a Standard No. 30 mesh screen (0.595 mm openings). All material remaining on the screen is washed into a plastic jar and preserved with buffered formalin containing Rose Bengal dye. Four replicate samples are collected at each sight. Each replicate is processed individually. Laboratory identification and enumeration of macro-benthic organisms in each sample is performed under contract by Hydrozoology Laboratory, Newcastle, CA 95658. Analysis has been done by Hydrozoology for the period of record. At the laboratory, the volume of settable substrate in each sample jar is estimated and recorded. The formalin fixative is poured off and the sample is collected on a 30-mesh screen. The composition of the substrate is estimated and recorded noting the relative percentages of peat, sand, mica, organic detritus, and other materials. The substrate is hand picked for organisms under a three diopter-illuminated magnifier. Organisms are placed in 70% ethyl alcohol for subsequent identification. A stereoscopic dissecting microscope (70-120x) is used to identify most organisms. When taxonomic features are too small for identification under the dissecting scope, the organism is permanently mounted on a slide and examined under a compound microscope. If more than four hours of picking is required, and a sample contains many organisms but few species, a one-fourth subsample is chosen at random. The subsample is picked and the results are multiplied by four to represent the total sample. The remainder of the sample is inspected to make sure no other taxa were overlooked. A multiplication factor of 19 is used to convert the number of organisms per grab sample to organisms per square meter using the following formula: 1.0 m^2 / 0.053 m^2 (sampling area of ponar) = 19. Enumerated taxa are recorded and the abundance data is entered and stored in the EMP benthos database. All organisms in an enumerated replicate sample are combined, preserved in alcohol, and archived by DWR.

Biomass in preserved, archived samples will be estimated using the "wet-weight" method as follows:

One randomly chosen replicate benthic sample from each historical sampling event at the four targeted sites will be sorted by species and separated into small petri dishes (before 1983, only one replicate sample was archived). Once all animals have been picked from the sample, each resulting monospecific subsample will be gently blotted dry on filter paper, a paper towel, or a similar absorbent surface for a consistent period of time to remove any excess fluid and weighed to the nearest microgram (Ricciardi and Bourget 1998, Wong 1998). It will be noted if the size distribution of each species is distinctly unimodal, bimodal, or composed of many year classes and these notes will be used in our data analyses. The separated subsamples will be preserved and stored in separate vials. Wet-weights of distinct taxa will be obtained by combining the weight of each specimen from a single sample. Mean individual weights will be obtained by dividing the combined data by the total number of organisms in the sample (Wong 1998). Population biomass and biomass per individual will be calculated using published wet-weight to tissue dry-weight conversion equations for individual taxa (Lie 1968, Ricciardi and Bourget 1998), and the dry-weight biomass will then be entered into the EMP benthos database alongside the historical and current abundance data. Sample processing will be conducted by EMP staff in consultation with Wayne Fields of Hydrozoology, the regional expert on benthos taxonomy. Organism weights in the preserved samples will have been somewhat altered because of the lengthy storage and the preservatives used. However, the preservation in alcohol after exposure to formalin has been shown to have little effect on wet-to-dry-weight conversion factors (Ricciardi and Bourget 1998) and the variability introduced by preservation and storage is probably negligible compared to the natural variability between replicate benthos samples.

Method tests will be conducted according to the tasks outlined in I. A. 2. a, above. To summarize these tasks briefly, we will compare the wet-weight, dry-tissue-weight, and constant factor methods for at least one species for which dry-weight data exists, test the validity of wet-weight to dry-weight conversion equations from literature sources on at least one SFE species for which dry-weight data exists, and examine the need for obtaining historical biomass for replicate samples. Since we will be working with historical samples and existing dry-weight data, no additional sampling is needed.

C. Data Reduction, Analysis and Reporting

We will estimate benthic biomass in historical samples using the wet-weight method outlined above and published wet to dry-weight conversion equations for individual taxa (Lie 1968, Ricciardi and Bourget 1998). We will compare results obtained with the wet-weight, dry-tissue-weight, and constant factor methods using analysis of variance and regression techniques. The effects of determining biomass in only one instead of three replicate samples will be determined via a power analysis (Hymanson et al. 1994) and in close cooperation with the sister project by Peterson et al. as part of their proposed retrospective analysis. Please note that the focus of this project is on providing crucial biomass data for the analyses proposed by Peterson et al. and for future analyses of the ecological role of benthic organisms (e.g., grazing, community metabolism, etc.), and on testing methods for obtaining reliable biomass estimates in preserved and future monitoring samples. Thus, this project does not itself include in-depth analyses.

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Figure 1: EMP Benthos Sampling Sites, 1977 - 2002. Blue: Current sampling sites. Red: Historically sampled sites. Circled: long-term sites targeted in this study.

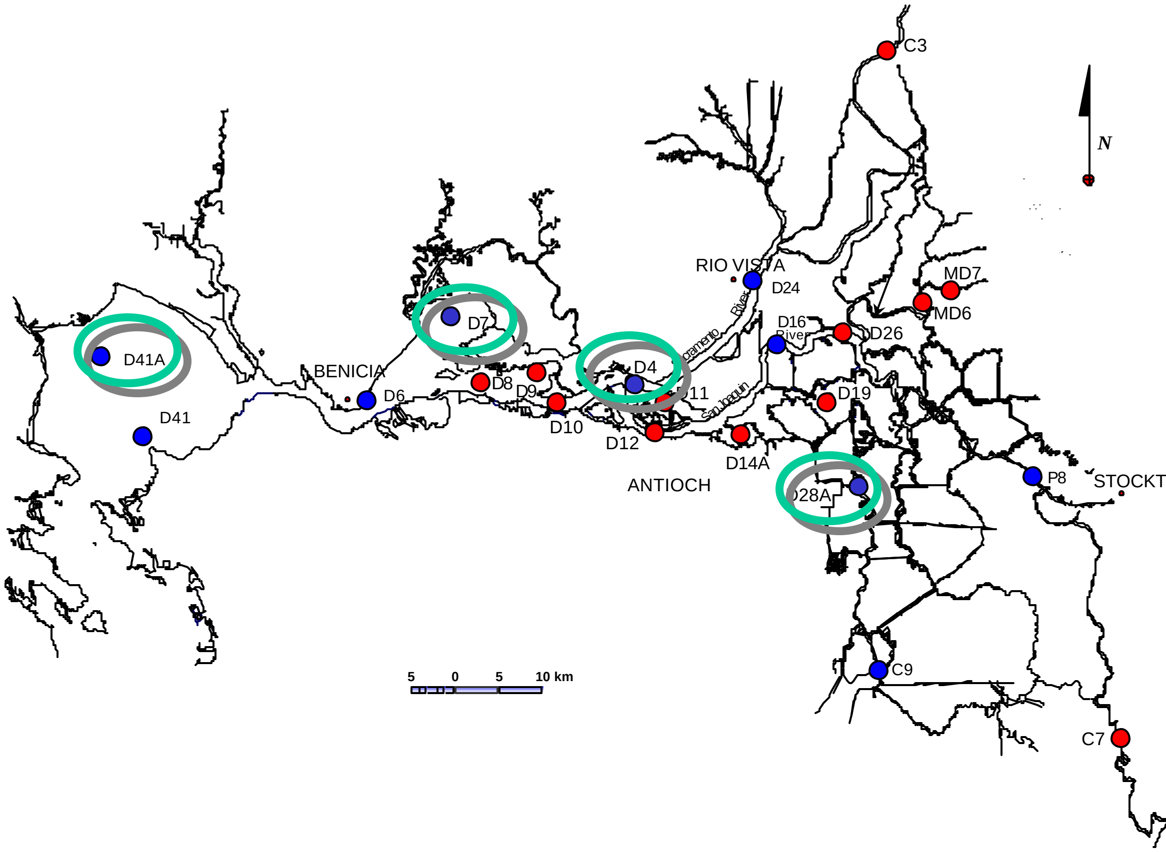


Figure 2: *Potamocorbula amurensis* abundance, dry tissue weight (biomass), and grazing rate at EMP station D7 (Grizzly Bay) in 1998 and 1999.

Abundance: left y-axis, pink circles and line, EMP data. Dry Tissue Weight: middle y-axis, calculated applying either multiple length to weight conversion factors based on monthly length and dry tissue weight measurements and monthly size frequency data, (blue circles and line, "by monthly equation," USGS data) or an estimate based on the average ash-free dry-weight/individual measured at D7 for *P. amurensis* from 1997 –2001and the abundance estimated for each month (pink squares and line, "by average afdw/animal," USGS data). Grazing Rates: right y-axis, calculated from known biomass-pumping rate relationships with a correction for a concentration boundary layer, lines and symbols same as for Dry Tissue Weight. Please see Appendix for more information on how these data were obtained. Figure courtesy of J. Thompson, USGS, and modified by A. Mueller-Solger.

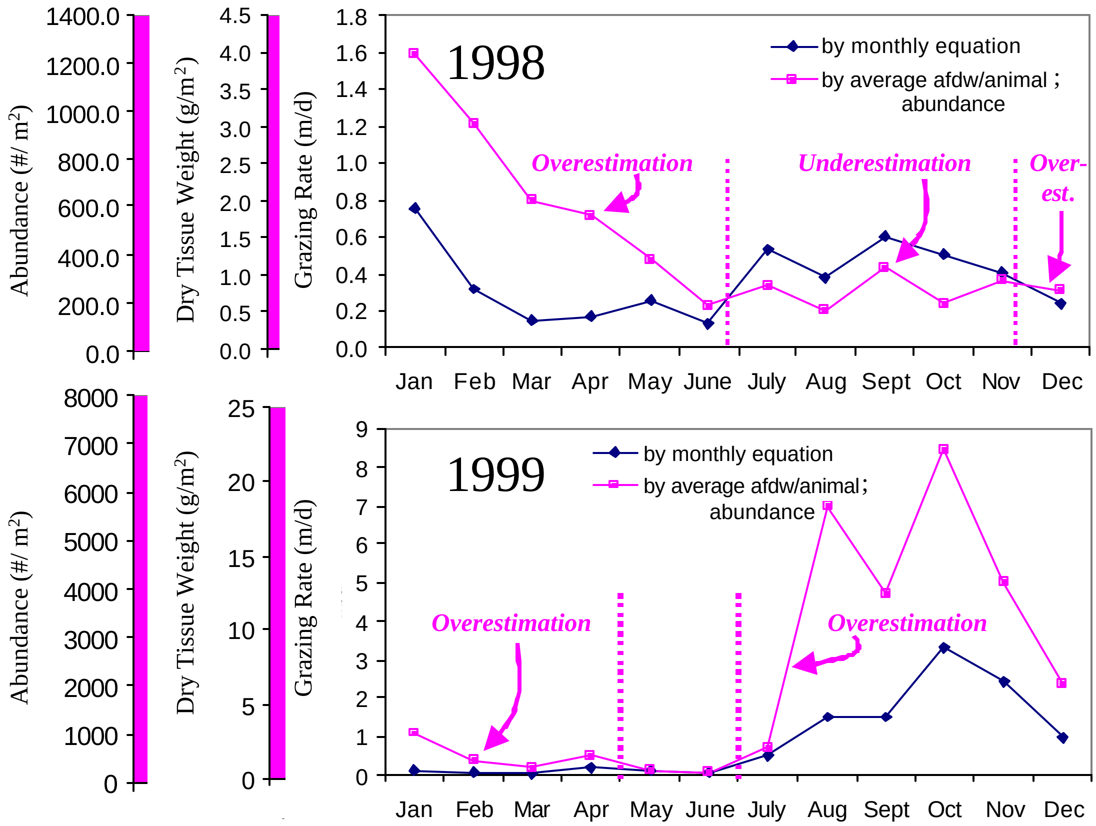
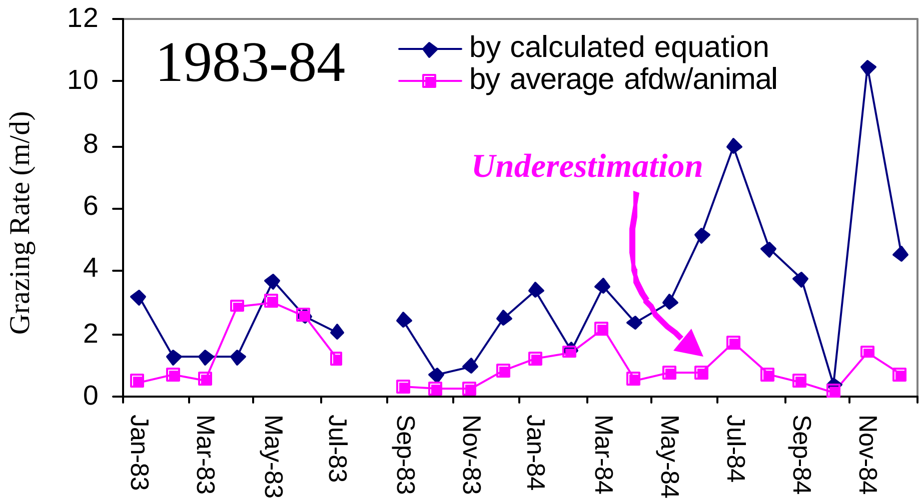


Figure 3: *Corbicula fluminea* grazing rates at EMP station D28A-L (Old River) in 1983 and 1984.

Grazing Rates: calculated from known biomass-pumping rate relationships with a correction for a concentration boundary layer. Population biomass was calculated by multiplying ash-free dry-weight (afdw) per animal with population abundance reported by the EMP. Ash-free dry-weight (afdw) per animal was obtained by applying either a general *Corbicula fluminea* length to weight regression equation derived from recent USGS *Corbicula* measurements in Mildred Island and Frank's Tract (blue circles and line, "by calculated equation," USGS data) or an estimate based on average *Corbicula* length at D28A-Left in 1983-1984 (Winternitz 1986, MS Thesis) and conversion to weight using the same regression equation as above (pink squares and line, "by average afdw/animal"). Please see Appendix for more information on how these data were obtained. Figure courtesy of J. Thompson, USGS, and modified by A. Mueller-Solger.



Appendix:

Methods to obtain benthos abundance, biomass, and grazing rate data (as used in Figures 2 and 3):

1. Abundance (=EMP benthos monitoring procedures):

This is the standard operating procedure for benthos community composition and abundance monitoring used by the EMP and described in the proposal, above. All abundance data shown in Figures 2 and 3 is EMP data. Biomass and grazing rate estimates are derived from this and additional data as explained in 2. and 3., below.

2. Biomass:

Population biomass for Potamocorbula amurensis and Corbicula fluminea was estimated in two ways: an "easy but inaccurate" way (a) and an "accurate but laborious" way (b). Both methods use one or multiple length-to-weight conversion equations. These equations are based on the relationship between measured length and measured dry tissue weight of non-preserved (live) animals collected by the USGS in conjunction with the EMP (*Potamocorbula*) or in a separate CALFED study (*Corbicula*). Neither of these methods is the wet-weight method we propose for this special study. As discussed in the proposal, the wet-weight method is intermediate in accuracy of results. While we have data from EMP stations (collected by the EMP, the USGS, and Leo Winternitz, see below) to show the difference in results using methods a) and b), we unfortunately do not have any wet-weight data and thus cannot show a comparison with the wet-weight method.

a) Constant factor method, "by average afdw/animal:" Ash-free dry-weight (afdw) of an average sized animal of each species was estimated based on established length to weight conversion equations (details for each species below), and then multiplied by the abundance of animals during each sampling event. This method assumes that biomass variability within each species is low and an average weight/animal thus suffices for abundance to biomass conversions. Since this assumption generally does not hold for benthic organisms, this method is considered the worst method to obtain benthic biomass and related variables.

b) Dry-tissue-weight method, "by … equation:" Lengths of each animal in each sample was measured and converted to weights using length to weight conversion equations for size classes grouped by 1mm increments and established for each month ("by monthly equation," Fig. 2) or for a longer time period ("by calculated equation," Fig. 3), see below for details. The weight of the animals in each size class was then multiplied by the number of animals in that size class. All size class weights were summed resulting in the total dry tissue weight for each sample. This method avoids assumptions and is recognized as the best method for obtaining benthic biomass and related variables. It is, however, very laborious and requires collection and swift processing of live specimens for establishing (preferably seasonal or monthly) length to weight conversion equations. As discussed in this proposal, this is not practical for routine EMP benthos monitoring and impossible for the historical preserved samples.

Details for each species:

Potamocorbula:

For a): The average dry tissue wt/animal was calculated from analyses of all live animals collected at D7 throughout a 5 year period from 1997 through 2001.

For b) Monthly equations for converting length to dry-weight were based on monthly regressions of the 1997-2001 length and weight data, above – e.g., all January data (all years combined) was regressed to obtain an "average" relationship for January. This was repeated for each month. The basic relationship is

$$\ln \text{dry wt (g)} = \text{intercept} + \text{x coefficient} * \ln \text{length (mm)}$$

Corbicula:

For a): Average dry tissue wt/animal based on average animal length (3.69mm) in L. Winternitz (1986, M.S. thesis) at D28A-Left during 1983-1984. This average length was converted to average weight with the same equation used in b), below.

For b): One general regression equation was used to convert *Corbicula* length to weight since appropriate data only exists for *Corbicula* samples collected in Frank's Tract and Mildred Island (J. Thompson, USGS) and not at the EMP site D28A. All available Frank's Tract and Mildred Island *Corbicula* data was combined to yield the general equation used for length to weight conversion "by calculated equation:"

$$\ln \text{dry wt (g)} = -11.84 + 3.2149 * \ln \text{length (mm)}$$

3. Grazing Rates:

Grazing rates include a concentration boundary layer adjustment (O'Riordan et al.1995) of pumping rates for *Corbicula* (Lauritsen 1986) and *Potamocorbula* (Cole et al.1992) and were calculated using biomass (calculated as above), abundance (EMP data), size of animals and a relationship that estimates the physiological pumping rate, independent of field conditions. The physiological pumping rate is reduced using a series of relationships that correct for the concentration of food most likely encountered by the animals in the field, resulting in "grazing rates." These corrections and resulting grazing rates were supplied and calculated by Jan Thompson, USGS.